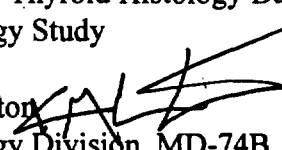OFFICE OF
RESEARCH AND DEVELOPMENT

CROFTON, 1998f

MEMORANDUM

Date: 22 October 1998 (revised 06 November 1998)

Subject: Analysis of the Thyroid Histology Data from the Rat Developmental
          Neurotoxicology Study

From: Kevin M. Crofton
          Neurotoxicology Division, MD-74B
          National Health Effects and Environmental Research Laboratory

To: Annie Jarabek
          National Center for Environmental Assessment

Attached is the statistical analysis of the morphometric histology data from the
Argus Rat Developmental Neurotoxicology Study (Argus Protocol #1613-002;
Study#7757A210-1096-25F). I have attached a description of how the analyses
were done and some summary graphs. Note that this contains only the from the
PND5 time point. The re-analysis of the PND90 time point data is outstanding
because I did not get adequate information until 10/27/98 and I just have not had
time to run the analysis. Note also that the data analyzed in this report are from
the final report for the PND5 timepoint (Channel, 1998b). The original data
sent from WPAFB (09/25/98) contained incorrect treatment codes which were
corrected in a final submission (Channel, 1998b).

Note: The raw data for this analysis is all contained in the Final Consultative
Letter submitted to the Agency (Channel, 1998b).

Analyses of Morphometric Histopathology Data from the Argus Developmental Neurotoxicology Study

Summary: The report from Argus Laboratories (York, 1998a) contains a Consultative Letter amendment (Channel, 1998a) entitled "Morphometric Analysis Report – Thyroid: A Neurobehavioral Developmental Study of Ammonium Perchlorate Administered Orally in Drinking Water to Rats". The following is a statistical analysis of data. Results of these re-analyses are similar to those stated in the report except that there were no gender related effects detected. There was a significant decrease in the lumen area measurement on PND5 in both the 3 and 10 mg/kg/day groups. Therefore, the NOAEL for thyroid histopathology based on the morphometric assessments is 1.0 mg/kg/day. These results are consistent with the known mechanism-of-action of perchlorate, i.e. inhibition of iodine uptake and decreased synthesis and release of thyroid hormone. The resulting increase in thyroid stimulating hormone will results in increased utilization of stored thyroid hormones and thus decreased lumen size.

Data Source: All data was supplied by Dr. William H. Baker, AFRL/HEST, Wright-Patterson AFB in Microsoft Excel spreadsheets. Data were exported to ascii format and used as input for SAS analyses. Data from the dependent measure (lumen area) were subjected to a three-way ANOVAs, with Gender (male and female), Treatment (dose), and Block (two separate analyses of separate blocks of data) as independent between-subjects variables. Step-down ANOVAs were conducted as indicated by significant interactions. Mean contrasts were performed using Turkey's Studentized Range (HSD) Test. SAS analysis code and output are attached.

Data Analysis - Results: There was a significant main effect of Treatment for the lumen area data for the 3 and 10 mg/kg/day group compared to controls. The data are plotted in Figures 1.

Notes: The original pilot morphometric study presented by Dr. Baker at a meeting at WPAFB in June 1998 contained measurements of both the follicular cell height and the lumen diameter (see also Appendix O of York, 1998a). In the final morphometric study an arbitrary decision was made by Dr. Baker to focus on only the lumen area measurement due to time limitations (phone conversation with Bill Baker). In the opinion of Dr. Charles Capen, Ohio University (phone conversation on 11/06/98) the measurement of follicular height is usually more sensitive than follicle diameter and lumen area. In support of this opinion, data collected by Dr. Baker (see Appendix O) demonstrated significant increases (in males rats) in the incidence of follicular epithelial cell hypertrophy at dosages much lower than those dosages that increased the incidence of decreased lumen area. Also, the subjective histopathology found in the 14-Day "Caldwell Study" shows a NOAEL for follicular cell hypertrophy at 0.3 mg/kg/day and a NOAEL for lumen area at 1.0 mg/kg/day (Channel, 1998a; AFRL-HE-CL-1998-0026). This suggests that the lumen area measurements may be underestimating the effects of perchlorate in the PND5 F1 animals. Therefore, I would suggest that we rely on the subjective assessments for relating thyroid hormone concentrations to thyroid histopathology as assessed by changes in follicular cells.
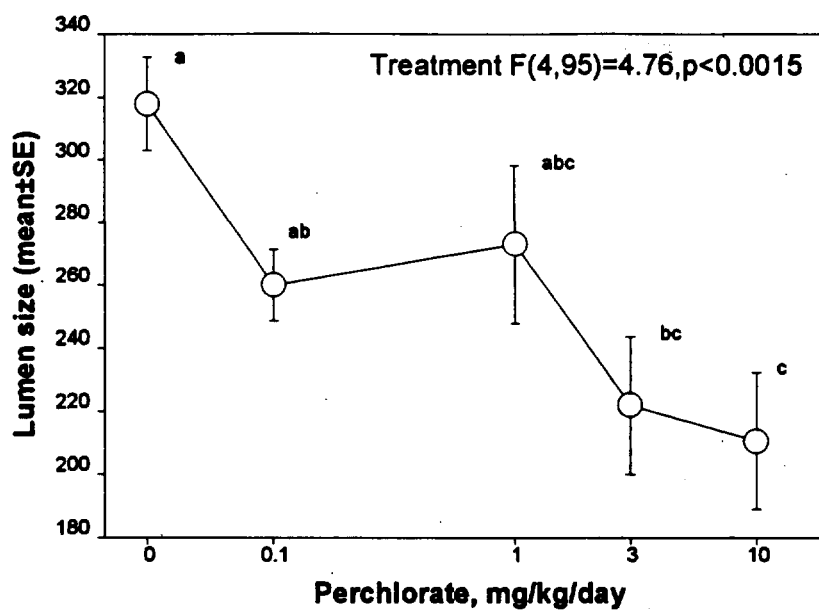
Figure 1. Effects of maternal perchlorate exposure on thyroid gland follicular lumen area in F1 generation offspring on postnatal day 5. Means with different letters were significantly different (p<0.05). Daily dose was estimated from water consumption data.

11   The SAS System   00:01
Monday, October 19, 1998

NOTE: Copyright (c) 1989-1996 by SAS Institute Inc., Cary, NC, USA.
NOTE: SAS (r) Proprietary Software Release 6.12 TS020
      Licensed to US ENVIRONMENTAL PROTECTION AGENCY, Site 0019614059.

NOTE: Running on ALPHASERVER Model 2100 5/300 Serial Number 80000000.

  Welcome to the NHEERL-RTP SAS Information Delivery System.

```
1          *THIS FILE IS HRLSAS[CROFTON.THYROID.PERCHLORATE]PERCHLORATE_PND5_MORPHOMETRICS.SAS;
2          *SAS CODE TO ANALYZE PUP THYROID MORPHOMETRIC DATA FROM;
3          *WPAFB-ARGUS DEVELOPMENTAL NEUROTOX STUDY OF PERCHLORATE;
4
5          DATA NEW; INFILE '[CROFTON.THYROID.PERCHLORATE]PERCHLORATE_PND5_THYROID_MORPHOMETRICS.TXT';
6          INPUT ANIMAL$ GENDER$ BLOCK DOSE AREA;
7
8
```

NOTE: The infile '[CROFTON.THYROID.PERCHLORATE]PERCHLORATE_PND5_THYROID_MORPHOMETRICS.TXT' is:
      File=DSA21:[SAS$USERS.CROFTON.THYROID.PERCHLORATE]PERCHLORATE_PND5_THYROID_MORPHOMETRICS.TXT

NOTE: 100 records were read from the infile
'[CROFTON.THYROID.PERCHLORATE]PERCHLORATE_PND5_THYROID_MORPHOMETRICS.TXT'.
      The minimum record length was 43.
      The maximum record length was 43.
NOTE: The data set WORK.NEW has 100 observations and 5 variables.

```
8          PROC SORT; BY DOSE;
9
```

NOTE: The data set WORK.NEW has 100 observations and 5 variables.

```
9          PROC PRINT; BY DOSE;
10
11
```

NOTE: The PROCEDURE PRINT printed pages 1-3.

```
11         PROC SORT; BY DOSE;
12
```

NOTE: Input data set is already sorted, no sorting done.

```
12         PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY DOSE;
13             VAR AREA;
```

```
14
15


NOTE: The PROCEDURE MEANS printed page 4.
15          PROC SORT; BY BLOCK DOSE;
16


NOTE: The data set WORK.NEW has 100 observations and 5 variables.
16          PROC MEANS N MEAN STDERR MIN MAX STD VAR CV; BY BLOCK DOSE;
17               VAR AREA;
18
```

\)

```
 19

NOTE: The PROCEDURE MEANS printed pages 5-6.

 19                PROC SORT; BY BLOCK DOSE GENDER;
 20
 21

NOTE: The data set WORK.NEW has 100 observations and 5 variables.

 21          PROC GLM;
 22              CLASSES BLOCK DOSE GENDER;
 23              MODEL AREA = BLOCK|DOSE|GENDER;
 24

NOTE: The PROCEDURE GLM printed pages 7-8.

 25                PROC GLM;
 26                    CLASSES DOSE;
 27                    MODEL AREA = DOSE;
 28                    MEANS DOSE/DUNCAN;
 29
 30

NOTE: The PROCEDURE GLM printed pages 9-11.

 30          PROC GLM; BY BLOCK;
 31              CLASSES DOSE;
 32              MODEL AREA = DOSE;
 33              MEANS DOSE/DUNCAN;
 34
 35          ENDSAS;

NOTE: The PROCEDURE GLM printed pages 12-17.

NOTE: SAS Institute Inc., SAS Campus Drive, Cary, NC USA 27513-2414
```

1   The SAS System   00:01 Monday,
October 19, 1998   1

--- DOSE=0

OBS	ANIMAL	GENDER	BLOCK	AREA
1	382	F	2	315.30
2	385	F	2	235.30
3	388	F	2	392.60
4	390	F	2	252.00
5	96B	F	1	258.10
6	97B	F	1	196.40
7	98B	F	1	418.70
8	99B	F	1	339.80
9	101B	F	1	364.20
10	102B	F	1	434.20
11	309	M	2	249.21
12	314	M	2	389.38
13	317	M	2	336.60
14	321	M	2	320.74
15	95A	M	1	361.14
16	96A	M	1	375.25
17	97A	M	1	307.62
18	98A	M	1	261.20
19	100A	M	1	257.78
20	101A	M	1	291.14

--- DOSE=0.3

OBS	ANIMAL	GENDER	BLOCK	AREA
21	365	F	2	199.10
22	369	F	2	226.30
23	371	F	2	221.70
24	374	F	2	216.60
25	85B	F	1	256.60
26	87B	F	1	303.80
27	88B	F	1	285.00
28	89B	F	1	419.30
29	93B	F	1	255.70
30	94B	F	1	262.70
31	297	M	2	250.13
32	301	M	2	193.47
33	303	M	2	203.19
34	306	M	2	271.87
35	85A	M	1	280.98
36	86A	M	1	256.36

```
37     87A        M          1        261.67
38     90A        M          1        303.47
39     91A        M          1        303.91
40     92A        M          1        225.23
```

--- DOSE=1

OBS	ANIMAL	GENDER	BLOCK	AREA
41	78B	F	1	248.3
42	79B	F	1	254.2

The SAS System

--- DOSE=1

                                                        (continued)

OBS	ANIMAL	GENDER	BLOCK	AREA
43	80B	F	1	405.90
44	81B	F	1	212.10
45	82B	F	1	228.90
46	83B	F	1	344.90
47	353	F	2	290.50
48	358	F	2	228.50
49	362	F	2	178.10
50	364	F	2	160.60
51	75A	M	1	294.17
52	76A	M	1	285.03
53	77A	M	1	200.61
54	80A	M	1	379.11
55	82A	M	1	370.13
56	84A	M	1	621.46
57	282	M	2	272.49
58	286	M	2	180.15
59	288	M	2	120.44
60	293	M	2	184.22

--- DOSE=3

OBS	ANIMAL	GENDER	BLOCK	AREA
61	68B	F	1	192.50
62	70B	F	1	341.20
63	71B	F	1	200.90
64	72B	F	1	230.50
65	73B	F	1	561.80
66	74B	F	1	172.30
67	338	F	2	244.30
68	342	F	2	184.50
69	344	F	2	118.60
70	350	F	2	109.30
71	67A	M	1	231.27
72	68A	M	1	191.71
73	69A	M	1	276.06
74	71A	M	1	208.06
75	72A	M	1	124.28

```
              76      74A        M          1        156.84
              77      270        M          2        267.16
              78      273        M          2        214.84
              79      278        M          2        223.47
              80      280        M          2        190.38
```

--- DOSE=10

OBS	ANIMAL	GENDER	BLOCK	AREA
81	58B	F	1	499.8
82	59B	F	1	214.1
83	61B	F	1	317.5

1   The SAS System   00:01 Monday,
October 19, 1998   3

-- DOSE=10
--.
                                                    (continued)

                OBS   ANIMAL   GENDER   BLOCK   AREA

                 84   64B   F   1   183.50
                 85   65B   F   1   244.40
                 86   66B   F   1   123.90
                 87   323   F   2   147.60
                 88   324   F   2   112.00
                 89   327   F   2   126.00
                 90   334   F   2   145.60
                 91   57A   M   1   115.60
                 92   58A   M   1   255.03
                 93   60A   M   1   163.76
                 94   62A   M   1   143.46
                 95   63A   M   1   214.62
                 96   65A   M   1   225.07
                 97   253   M   2   270.34
                 98   257   M   2   360.41
                 99   259   M   2   139.56
                100   265   M   2   209.18

1   The SAS System   00:01 Monday,
October 19, 1998   4

Analysis Variable : AREA

-- DOSE=0

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	20	317.8330000	14.9028002	196.4000000	434.2000000	66.6473488	4441.87
20.9692979							

-- DOSE=0.3

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	20	259.8540000	11.4163555	193.4700000	419.3000000	51.0554937	2606.66
19.6477613							

-- DOSE=1

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	20	272.9905000	25.1889912	120.4400000	621.4600000	112.6485932	12689.71
41.2646569							

-- DOSE=3

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
	20	221.9985000	21.7542975	109.3000000	561.8000000	97.2881760	9464.99
43.8237988							

-- DOSE=10

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
	20	210.5715000	21.6752513	112.0000000	499.8000000	96.9346706	9396.33
46.0340885							

1   The SAS System   00:01 Monday,
October 19, 1998   5

Analysis Variable : AREA

-- BLOCK=1 DOSE=0

| | N | Mean | Std Error | Minimum | Maximum | Std Dev | Variance |
CV							
	12	322.1275000	20.8245302	196.4000000	434.2000000	72.1382886	5203.93
22.3943279							

-- BLOCK=1 DOSE=0.3

| | N | Mean | Std Error | Minimum | Maximum | Std Dev | Variance |
CV							
	12	284.5600000	14.0889565	225.2300000	419.3000000	48.8055768	2381.98
17.1512429							

-- BLOCK=1 DOSE=1

| | N | Mean | Std Error | Minimum | Maximum | Std Dev | Variance |
CV							
	12	320.4008333	33.7592013	200.6100000	621.4600000	116.9453036	13676.20
36.4996877							

-- BLOCK=1 DOSE=3

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	12	240.6183333	33.4036403	124.2800000	561.8000000	115.7136042	13389.64
48.0901029							

--- BLOCK=1 DOSE=10

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	12	225.0616667	30.1369216	115.6000000	499.8000000	104.3973588	10898.81
46.3861129							

--- BLOCK=2 DOSE=0

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	8	311.3912500	21.7940734	235.3000000	392.6000000	61.6429483	3799.85
19.7959796							

--- BLOCK=2 DOSE=0.3

	N	Mean	Std Error	Minimum	Maximum	Std Dev	Variance
CV							
	8	222.7950000	9.4615619	193.4700000	271.8700000	26.7613383	716.1692286
12.0116422							

1   The SAS System   00:01 Monday,
October 19, 1998   6

                    Analysis Variable : AREA

        -- BLOCK=2 DOSE=1

                N   Mean   Std Error   Minimum   Maximum   Std Dev   Variance
CV

        ----
                8   201.8750000   20.3689056   120.4400000   290.5000000   57.6119650   3319.14
28.5384347

        -- BLOCK=2 DOSE=3

                N   Mean   Std Error   Minimum   Maximum   Std Dev   Variance
CV

        ----
                8   194.0687500   19.8871049   109.3000000   267.1600000   56.2492269   3163.98
28.9841754

        -- BLOCK=2 DOSE=10

                N   Mean   Std Error   Minimum   Maximum   Std Dev   Variance
CV

        ----
                8   188.8362500   30.5732245   112.0000000   360.4100000   86.4741375   7477.78
45.7931872

        ----

1   The SAS System   00:01 Monday,
October 19, 1998   7

General Linear Models Procedure
Class Level Information

Class	Levels	Values
BLOCK	2	1 2
DOSE	5	0 1 3 10 0.3
GENDER	2	F M

Number of observations in data set = 100

1   The SAS System   00:01 Monday,
October 19, 1998   8

General Linear Models Procedure

Dependent Variable: AREA

Source	DF	Sum of Squares	Mean Square	F Value
Pr > F				
Model	19	346209.90265000	18221.57382368	2.73
0.0010				
Error	80	534073.52542501	6675.91906781	
Corrected Total	99	880283.42807501		

	R-Square	C.V.	Root MSE
AREA Mean			
	0.393294	31.83575	81.70629760
256.64950000			

Source	DF	Type I SS	Mean Square	F Value
Pr > F				
BLOCK	1	71968.87760417	71968.87760417	10.78
0.0015				
DOSE	4	146891.83317000	36722.95829250	5.50
0.0006				
BLOCK*DOSE	4	31028.08228833	7757.02057208	1.16
0.3339				
GENDER	1	74.73602500	74.73602500	0.01
0.9160				
BLOCK*GENDER	1	25175.45150417	25175.45150417	3.77
0.0557				
DOSE*GENDER	4	10590.60917000	2647.65229250	0.40
0.8105				
BLOCK*DOSE*GENDER	4	60480.31288833	15120.07822208	2.26
0.0694				

Source	DF	Type III SS	Mean Square	F Value
Pr > F				
BLOCK	1	71968.87760417	71968.87760417	10.78
0.0015				

Source	DF			F	Pr > F
DOSE	4	144140.98140433	36035.24535108	5.40	0.0007
BLOCK*DOSE	4	31028.08228833	7757.02057208	1.16	0.3339
GENDER	1	541.17704817	541.17704817	0.08	0.7766
BLOCK*GENDER	1	25175.45150417	25175.45150417	3.77	0.0557
DOSE*GENDER	4	4930.14328433	1232.53582108	0.18	0.9458
BLOCK*DOSE*GENDER	4	60480.31288833	15120.07822208	2.26	0.0694

1
October 19, 1998   9

The SAS System

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	5	0 1 3 10 0.3

Number of observations in data set = 100

1   The SAS System   00:01 Monday,
October 19, 1998   10

General Linear Models Procedure

Dependent Variable: AREA

Source	DF	Sum of Squares	Mean Square	F Value
Pr > F

| Model | 4 | 146891.83317000 | 36722.95829250 | 4.76 |
0.0015

| Error | 95 | 733391.59490501 | 7719.91152532 | |

| Corrected Total | 99 | 880283.42807501 | | |

| R-Square | C.V. | Root MSE |
AREA Mean

| 0.166869 | 34.23464 | 87.86302707 |
256.64950000

Source	DF	Type I SS	Mean Square	F Value
Pr > F

| DOSE | 4 | 146891.83317000 | 36722.95829250 | 4.76 |
0.0015

Source	DF	Type III SS	Mean Square	F Value
Pr > F

| DOSE | 4 | 146891.83317000 | 36722.95829250 | 4.76 |
0.0015

1

00:01 Monday,

October 19, 1998   11

General Linear Models Procedure

Duncan's Multiple Range Test for variable: AREA

NOTE: This test controls the type I comparisonwise error rate, not the

experimentwise error rate

Alpha= 0.05   df= 95   MSE= 7719.912

Number of Means	2	3	4	5
Critical Range	55.16	58.05	59.96	61.36

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	DOSE
	A		317.83	20	0
	A				
B	A		272.99	20	1
B	A				
B	A	C	259.85	20	0.3
B		C			
B		C	222.00	20	3
		C			
		C	210.57	20	10

1   The SAS System   00:01 Monday,
October 19, 1998   12

- BLOCK=1
- -

General Linear Models Procedure
Class Level Information

Class   Levels   Values

DOSE   5   0 1 3 10 0.3

Number of observations in by group = 60

--- BLOCK=1

General Linear Models Procedure

Dependent Variable: AREA

Source	DF	Sum of Squares	Mean Square	F Value
Pr > F				
Model	4	95837.08714333	23959.27178583	2.63
0.0440				
Error	55	501056.24545000	9110.11355364	
Corrected Total	59	596893.33259334		

R-Square	C.V.	Root MSE
AREA Mean		
0.160560	34.26518	95.44691485
278.55366667		

Source	DF	Type I SS	Mean Square	F Value
Pr > F				
DOSE	4	95837.08714333	23959.27178583	2.63
0.0440				

Source	DF	Type III SS	Mean Square	F Value
Pr > F				
DOSE	4	95837.08714333	23959.27178583	2.63
0.0440				

1   The SAS System   00:01 Monday,
October 19, 1998   14

-- BLOCK=1

General Linear Models Procedure

Duncan's Multiple Range Test for variable: AREA

NOTE: This test controls the type I comparisonwise error rate, not the
experimentwise error rate

Alpha= 0.05   df= 55   MSE= 9110.114

Number of Means   2   3   4   5
Critical Range   78.09 82.14 84.81 86.75

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	DOSE
	A	322.13	12	0
	A			
	A	320.40	12	1
	A			
B	A	284.56	12	0.3
B	A			
B	A	240.62	12	3
B				
B		225.06	12	10

The SAS System

    --- BLOCK=2

                              General Linear Models Procedure
                                 Class Level Information

                         Class   Levels   Values

                         DOSE   5   0 1 3 10 0.3

                    Number of observations in by group = 40

1   The SAS System   00:01 Monday,
October 19, 1998   16

-- BLOCK=2

General Linear Models Procedure

Dependent Variable: AREA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	82082.82831500	20520.70707875	5.55	0.0014
Error	35	129338.38956250	3695.38255893		
Corrected Total	39	211421.21787750			

R-Square	C.V.	Root MSE	AREA Mean
0.388243	27.16331	60.78965832	223.79325000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	4	82082.82831500	20520.70707875	5.55	0.0014

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	4	82082.82831500	20520.70707875	5.55	0.0014

1   The SAS System   00:01 Monday,
October 19, 1998   17

-- BLOCK=2

General Linear Models Procedure

Duncan's Multiple Range Test for variable: AREA

NOTE: This test controls the type I comparisonwise error rate, not the
experimentwise error rate

Alpha= 0.05   df= 35   MSE= 3695.383

Number of Means   2   3   4   5
Critical Range   61.70 64.87 66.92 68.40

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	DOSE
A	311.39	8	0
B	222.80	8	0.3
B			
B	201.88	8	1
B			
B	194.07	8	3
B			
B	188.84	8	10